Regulation of Glucose Transport in the NIH 3T3 L1 Preadipocyte Cell Line by TCDD

Hugh Olsen, Essam Enan, and Fumio Matsumura

Department of Environmental Toxicology and Institute of Toxicology and Environmental Health, University of California-Davis, Davis, CA 95620 USA

This study examined the changes in cellular glucose uptake induced by 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) as measured by quantification of intracellular radioactivity in the NIH 3T3 L1 preadipocyte cell line after a 30-minute incubation with the nonmetabolizable radioactive analogue of glucose, 3-*O*-methyl-D-[1-^{3}H] glucose. Treatment of differentiated NIH 3T3 L1 cells with TCDD produced a time- and dose-dependent decrease in the cellular uptake of glucose. Treatment of cells for 3 hr with 10^{-8} M TCDD significantly reduced glucose uptake to about 10% of control values ($p \leq 0.05$). Furthermore, cytochalasin B, a specific inhibitor of facilitative glucose transporter proteins totally abolished the portion of glucose transport activity that is sensitive to TCDD. The role of the Ah receptor in TCDD-mediated reduction in glucose uptake was investigated. Pretreatment of 3T3 L1 cells with the Ah receptor blocker 4,7-phenanthroline antagonized the effects of TCDD on glucose uptake. Structure–activity relationship studies with TCDD and two polychlorinated biphenyl (PCB) congeners revealed a rank order for their potency in the inhibition of glucose transport as follows: TCDD <<3,3′,4,4′ tetrachlorobiphenyl <2,2′,5,5′ tetrachlorobiphenyl (TCB). Such a rank order correlates both with previously determined biological activity of TCDD and the more active 3,3′,4,4′- and less active 2,2′,5,5′- TCB and with affinity for binding to the Ah receptor. The thyroid hormone T_4, like TCDD, reduced glucose uptake and blocked the action of TCDD to further reduce glucose uptake. Experimental evidence is consistent with a proposed mechanism for TCDD to reduce the titer of functional glucose transporter proteins through its interaction with the Ah receptor. *Key words:* cytochalasin B, dioxin, glucose transporter, glucose uptake, PCBs, TCDD. *Environ Health Perspect* 102:454–458 (1994)

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic congener of a large class of toxic pollutants, collectively known as halogenated dioxins (*1,2*). The dioxins, together with the halogenated dibenzofurans, polychlorinated biphenyls, and polybrominated biphenyls, belong to a larger class of toxins collectively referred to as the halogenated aromatic hydrocarbons (HAHs). HAHs are characterized by a common set of toxic effects and biochemical changes, including immunosuppression, carcinogenesis, teratogenesis, and induction of cytochrome CYP1A1 and other components of xenobiotic detoxification enzyme systems. One of the most prominent symptoms of poisoning in many species is a marked loss of body weight called "wasting syndrome." In the guinea pig this wasting syndrome appears to be directly correlated to the lethal effects of TCDD (LD_{50} for males, 0.6 µg/kg) (*3*). Associated with this wasting syndrome is a decrease in food intake and indications of malnourishment. Nevertheless, poisoned animals retain normal patterns of carbohydrate, lipid, and protein metabolism (*4*). Initially it was hypothesized that TCDD reduced intestinal absorption of nutrients, and it was assumed that reduction of absorbed nutrients was responsible for weight loss. Nevertheless, administration of parenteral nutrition to poisoned animals, although increasing body weight, did not prevent them from dying (*5*). Reduction of caloric intake in untreated animals to produce weight loss matching that of TCDD-treated animals did not produce the lethal effects seen in TCDD-poisoned animals (*6*). Peterson et al. (*4*) concluded that treated animals acquired a lower "set point" for maintenance of their body weight.

Recently we reported that TCDD inhibits glucose uptake in guinea pig adipose and guinea pig pancreatic tissue, both *in vivo* and *in vitro*, using isolated adipose and pancreas tissue culture (*7,8*). The inhibition of glucose uptake in adipose cells provides a plausible explanation for the loss of adipose tissue *in vivo* in TCDD-induced wasting syndrome. Adipose tissue contains low levels of glycerol kinase, which has been estimated in humans as being able to convert only 2.5–3.5% of the glycerol released from triglyceride breakdown and turnover into the 3-phosphoglycerol necessary for the reesterification of fatty acids to triglycerides (*9*). The breakdown of glucose to generate 3-phosphoglycerol, therefore, serves as the key rate-limiting reaction for the overall lipogenic process in adipose tissue, with a loss of glucose uptake making the reesterification of fatty acids impossible, thus leading to the loss of stored triglycerides. Because of the potential importance of reduced glucose uptake in TCDD-generated wasting syndrome, we have extended these observations in the guinea pig to another TCDD-sensitive species, the mouse, while establishing an *in vitro* cultured cell model for dioxin effects that is more convenient for experimental manipulation. We found the NIH 3T3 L1 preadipocyte cell line contains a cytosolic protein whose properties correspond to those of the Ah receptor based on results in sucrose density-gradient experiments with tritiated TCDD and DNA gel shift experiments using a dioxin responsive element (DRE) (unpublished data). We therefore examined glucose transport in the NIH 3T3 L1 adipocyte cell line and found that TCDD inhibits glucose transport in a dose- and time-dependent manner. The facilitated diffusion of D-glucose across cell membranes is mediated by a set of stereospecific transport proteins referred to as "GLUTs." Two glucose transporters, GLUT 1 and GLUT 4, are expressed in the NIH 3T3 L1 preadipocyte cell line (*10*) and are good candidates for regulation by TCDD.

Materials and Methods

We purchased 3-*O*-methyl-D-[1-^{3}H] glucose (^{3}H-Me-glc) (2.74 and 2.34 Ci/mmol) from Amersham (Arlington Heights, IL). Dulbecco's phosphate-buffered saline without Ca^{+2} and Mg^{+2}, calf serum, and antibiotics were purchased from Gibco/BRL (Grand Island, NY). All other biochemicals were purchased from Sigma Chemical Co. (St Louis, MO). TCDD was donated to the laboratory by Dow Chemical Co. (Midland, MI), and the polychlorinated biphenyl congeners were kindly donated by S. Safe (Texas A&M).

We obtained the NIH 3T3 L1 preadipocyte cell line from ATCC (culture no. CCL 92.1). Cells were cultured and differentiated as described by Student et al. (*11*), with minor modifications. Cells were passaged three times without being allowed to reach confluence and then frozen in aliquots of 10^7 cells/vial for future use. For any particular experiment, a frozen aliquot

Address correspondence to F. Matsumura, Institute of Toxicology and Environmental Health, University of California, Davis, CA 95620 USA.

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of cells was grown to confluence on 60-mm dishes in a 5% CO_2 atmosphere in a basal medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) high glucose without sodium pyruvate (Gibco, Grand Island, NY), 10% calf serum, 3.7 mg/ml sodium bicarbonate, 1000 U/ml penicillin G, and 100 µg/ml streptomyosin. We then cultured the cells for three more days in a differentiating medium that consisted of the basal medium plus 10 µg/ml insulin, 0.5 mM methylisobutyl xanthine, and 0.25 µM dexamethasone. The medium was then replaced with the maintenance medium which consisted of the basal medium containing 10 µg/ml insulin, and the cells were maintained for 2–10 days before being used in experiments. We treated cells with TCDD or PCB congeners for specified periods of time by adding test compounds in acetone to the maintenance medium. The volume of test compound added was 0.1% of total volume of media. We changed maintenance medium every 3 days. There were approximately 1.5×10^6 cells per 60-mm dish after differentiation.

Glucose uptake was measured using the methods of Foley et al. (*12*) and Horuk et al. (*13*), with minor changes. The media used for the glucose uptake assays was DMEM/no glucose (Gibco/BRL, Grand Island, NY), supplemented with 4.026 g/l sodium pyruvate, 0.584 g/l glutamate, and 3.7 g/l sodium bicarbonate. Medium was prepared, adjusted to pH 7.4, and filter sterilized before use. After treatment with test compounds or vehicle the cell monolayers were washed twice with DMEM/no glucose. The cells were then incubated at 37°C for 30 min with 2 ml DMEM/no glucose supplemented with 1 µCi/mL of 3-*O*-methyl-D-[1-^{3}H] glucose (^{3}H-Me-glc) and specified concentrations of unlabeled D-glucose. We then washed the cell monolayers two times with DMEM/no glucose, collected the cell monolayer with a cell scraper in 0.4 ml of 1.0% w/v sodium dodecyl sulfate, and quantified radioactivity by liquid scintillation counting using Scint-A XF aqueous compatible liquid scintillation solution, (Packard Instrument Co., Meriden, CT). Determination of disintegrations per minute was made based on quench curves.

After initial experiments (Table 1), glucose uptake assays included determination of ^{3}H-Me-glc nonspecific binding using a susceptibility test for cytochalasin B (Sigma, St Louis, MO) as follows. Cytochalasin B was administered to the cell monolayers by adding of 70 µg of cytochalasin B in 10 µl of absolute ethanol to each 60-mm plate 4 hr before glucose uptake assays. We added test compounds to the media 1 hr later (3 hr before glucose uptake assays) as described above. The portion of ^{3}H-Me-glc uptake that was inhibited by this concentration of cytochalasin B was defined as the uptake activity through the glucose transporter proteins.

Cells were seeded on 60-mm plates and differentiated as described above. They were subsequently treated for 72 hr with either hormone alone or hormone plus 10^{-8} M TCDD. Hormone concentrations in maintenance medium were 1 ng/ml tetraiodothyronine (T_4), 10 ng/ml triiodothyronine (T_3), 10 ng/ml tumor necrosis factor-α (TNFα), and 1 µg/ml of antibody against TNFα. Glucose uptake assays were performed as described above.

Table 1. Total glucose uptake at various glucose concentrations in NIH 3T3 L1 cells treated with 10^{-8} M TCDD for 24 hr

D-Glucose concentration (mM)	Glucose uptake (pg 3-*O*-methyl-D-[1-^{3}H] glucose/1.5 million cells)[a]		
	Control	TCDD[b]	TCDD effect (% of control)
0	273.0 ± 25.6	252.0 ± 33.1	92.3
1.6	181.8 ± 29.3	220.0 ± 39.4	121.0
3.3	205.9 ± 14.8	198.1 ± 22.4	96.2
6.7	172.8 ± 5.7	155.9 ± 4.7[c]	90.2
13.3	178.0 ± 43.1	145.0 ± 27.6	81.5

[a]30-min incubation; means ± 1 SD; $n = 3$.
[b]When data are pooled for all control versus TCDD-treated values, TCDD-treated cells had less ^{3}H-Me-glc uptake than controls ($p \leq 0.01$, two-tailed paired Student's *t*-test).
[c]For the 6.7 mM glucose concentration, TCDD's effect on ^{3}H-Me-glc uptake was significantly decreased compared to the corresponding control ($p \leq 0.05$, two-tailed Student's *t*-test).

Table 2. Inhibition of glucose transport by TCDD in differentiated and undifferentiated NIH 3T3 L1 preadipocytes

State of cell[a]	Glucose uptake (pg ^{3}H-methyl-glucose/1.5 million cells)[b]					
	Control			TCDD		
	Alone	+Cyto-B[c]	Net specific uptake[d]	Alone	+Cyto-B[c]	Net specific uptake[d]
Differentiated	102.0 ± 10.3	73.0 ± 7.2	29.0	55.8 ± 7*	52.7 ± 4.6	3.1 (10% of control)
Undifferentiated	48.3 ± 3.1	22.0 ± 1.0	26.2	43.8 ± 5.2	27.9 ± 5.4	15.9 (61% of control)

[a]Differentiated cells were treated for 72-hr period with media containing 0.25 µM dexamethasone, 10 µg/ml insulin, and 0.5 mM methylisobutyl xanthene. Undifferentiated cells were grown to confluence in DMEM high glucose with 10% calf serum. Lipisomes are evident in the cells after treatment with the differentiating media.
[b]30-min incubation; means ± 1 SD; $n = 3$.
[c]For experiments involving cyto-B (cytochalasin B), 70 µg of cytochalasin B in 10 µl of ethanol were added to each dish of cells and incubated for 1 hr before addition of TCDD in acetone or acetone alone (control) and then incubated for 3 hr before assay for glucose transport.
[d]Glucose uptake assays employed 1 µCi of ^{3}H-Me-glc and 13.3 mM cold glucose. Net uptake of ^{3}H-Me-glc is the nonspecific binding of ^{3}H-Me-glc determined by addition of cytochalasin B subtracted from corresponding control or TCDD values.
*Significantly different from control at $p \leq 0.05$ (two-tailed Student's *t*-test).

Results

Table 1 is a tabulation of the results of initial experiments to determine the inhibitory effect of TCDD on glucose uptake in NIH 3T3 L1 cells using various concentrations of unlabeled D-glucose in the glucose uptake assay. After 24 hr of treatment with 10^{-8} M TCDD, treated cells showed significantly decreased uptake of ^{3}H-Me-glc compared to control (for all controls versus all treated cells, $p \leq 0.01$, two-tailed, paired Student's *t*-test). When comparing effects of TCDD at individual glucose concentrations, a statistically significant reduction in glucose uptake occurred at the 6.7 mM D-glucose concentration ($p \leq 0.05$, two-tailed Student's *t*-test). Furthermore, there is a clear trend for the effect of TCDD on glucose uptake to become more pronounced with increasing concentrations of unlabeled D-glucose. Based on these results, a physiologically relevant concentration of 13.3 mM unlabeled D-glucose was selected for remaining uptake assays.

These initial assays (Table 1) quantified the total amount of ^{3}H-Me-glc recovered with the L1 cells after washing and include a significant portion of ^{3}H-Me-glc nonspecific binding. The portion of ^{3}H-Me-glc nonspecific binding was determined by pretreating some replicates of L1 cells with cytochalasin B, a specific inhibitor of facilitative glucose transporters (*14*), before measuring uptake of ^{3}H-Me-glc. These experimental results are tabulated in Table 2. From about 50% to about 90% of the

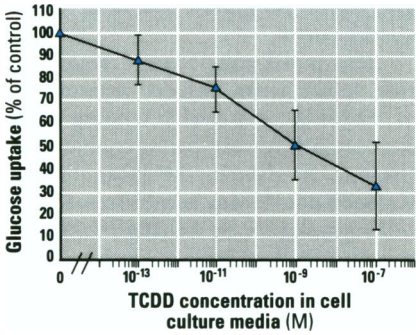

Figure 1. Dose response for the inhibition of glucose uptake by TCDD after a 3-hr exposure. Glucose uptake assays employed 1 μCi of ^{3}H-Me-Glc and 13.3 mM cold glucose. Net uptake of ^{3}H-Me-glc is the nonspecific binding of ^{3}H-Me-glc determined by addition of cytochalasin B subtracted from corresponding control or TCDD values. Vertical bars indicate the range of standard error. Statistically significant decrease in uptake occurred starting at 10^{-9} M TCDD ($p \leq 0.05$, Student's *t*-test).

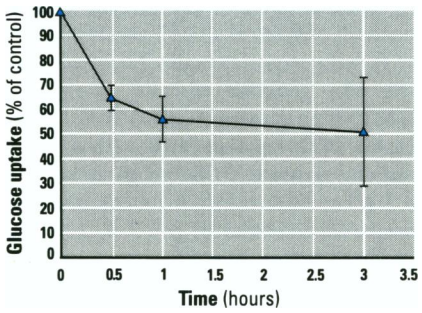

Figure 2. Time course for the inhibition of glucose uptake by TCDD. Glucose uptake assays employed 1 μCi of ^{3}H-Me-glc and 13.3 mM cold glucose. Net uptake of ^{3}H-Me-glc is the nonspecific binding of ^{3}H-Me-glc determined by addition of cytochalasin B subtracted from corresponding control or TCDD values. Cells were treated with 10^{-8} M TCDD for specified times before glucose uptake assays. Vertical bars indicate the range of standard error. Statistically significant decrease in uptake occurred starting at 30 min ($p \leq 0.05$, student's *t*-test).

Table 3. Effect of the Ah receptor blocker 4,7-phenanthroline on TCDD-mediated reduction of glucose transport

Assay conditions[a]	Glucose uptake (pg ^{3}H-methyl-glucose/1.5 million cells)[b] Without cytochalasin B	With cytochalasin B	Net specific uptake
Control (acetone vehicle)	432.2 ± 59.1	372.2 ± 53.8	60.0
10^{-8} M TCDD (3-hr incubation)	343.4 ± 22.2*	337.5 ± 35.1	5.9 (9.8% of control)
4,7-Phenanthroline	526.3 ± 18.8*	372.2 ± 53.8[c]	154.1
4,7-Phenanthroline + 10^{-8} M TCDD (3-hr incubation)	467.5 ± 60.8	337.5 ± 35.1[c]	130.0 (84.4% of 4,7-phenanthroline control)

[a]Glucose assays employed 1 μCi ^{3}H-Me-glc plus 13.3 mM cold glucose. Cells were incubated with or without 10 μM 4,7-phenanthroline for 1 hr, followed by addition of vehicle control or TCDD in acetone and incubation for 3 hr. Glucose assays were then performed as described in materials and methods using 13.3 mM cold D-glucose. Nonspecific binding of ^{3}H-Me-Glc was determined by adding cytochalasin B to cell cultures as described in Materials and Methods.
[b]30-min incubations; means ± 1 SD; $n = 5$.
[c]Used average value for nonspecific binding of ^{3}H-Me-Glc based on cytochalasin B value either from control or TCDD-treated cells.
*Significantly different from controls at $p \leq 0.05$ (two-tailed Student's *t*-test).

Table 4. Comparison of the effects of TCDD and diffferent PCB congeners on reduction of glucose transport in NIH 3T3 L1 preadipocytes

Compound tested[a]	Glucose uptake (pg ^{3}H-methyl-glucose/1.5 million cells)[b] With cytochalasin B	Without cytochalasin B	Net specific uptake
Control (acetone vehicle)	137.5 ± 36.7	73.3 ± 35.4	64.2
10^{-8} M TCDD (3-hr incubation)	110.2 ± 26.1	103.6 ± 47.9	6.6 (10.3% of control)
10^{-7} M 3,3',4,4'-Tetrachlorobiphenyl (3-hr incubation)	104.7 ± 11.7	85.9 ± 20.9	18.8 (29.3% of control)
10^{-7} M 2,2',5,5'-Tetrachlorobiphenyl (3-hr incubation)	117.2 ± 16.7	90.7 ± 30.0	26.5 (41.3% of control)

[a]Glucose assays employed 1 μCi ^{3}H-Me-glc plus 13.3 mM cold glucose. Cells were incubated with or without 70 μg cytochalasin B for 1 hr, followed by addition of vehicle control, TCDD, or PCB congeners in methanol and incubation for 3 hr. Glucose assays were then performed as described in Materials and Methods. Nonspecific binding of ^{3}H-Me-glc was determined by addition of cytochalasin B, and glucose uptake determined as the difference between total disintegrations per minute and disintegrations per minute with cytochalasin B treatment.
[b]30-min incubations; means ± 1 SD; $n = 4$.

total radioactivity recovered is ^{3}H-Me-glc nonspecific binding. Accordingly, TCDD-mediated inhibition of glucose uptake changes from about 10% for data not corrected for nonspecific binding to about 90% for data corrected for nonspecific binding (Table 2). TCDD significantly reduced glucose uptake ($p \leq 0.05$, two-tailed Student's *t*-test) for differentiated cells. For undifferentiated 3T3 L1 cells the magnitude of TCDD-mediated reduction in glucose was less and was not significant at the $p \leq 0.05$ level.

Figure 1 shows the results of a representative experiment to determine the dose response for TCDD-mediated inhibition of glucose uptake after a 3-hr incubation of 3T3 L1 cells with various concentrations of TCDD. Glucose uptake assays included 13.3 mM cold D-glucose. Data are presented as percentage of control ± 1 SE ($n = 5$) and have been corrected for nonspecific binding of ^{3}H-Me-glc. The control value was 29.5 pg ^{3}H-Me-glc/1.5 million cells/30-min incubation. A clear decline in specific glucose uptake with dose is evident, which becomes statistically significant at 10^{-9} M ($p \leq 0.05$, two-tailed Cochran *t*-test).

Figure 2 depicts the results of a representative experiment on the time course of inhibition of glucose transport by incubation of 3T3 L1 cells with 10^{-8} M TCDD. Results are the average of four replicates ± 1 SE and have been corrected for nonspecific binding of ^{3}H-Me-glc and displayed as percentage of control. The time-zero control value is 46.7 pg ^{3}H-Me-glc/1.5 million cells/30-min incubation. Glucose uptake assays included 13.3 mM cold D-glucose. The TCDD-induced decline in glucose transport is significant ($p \leq 0.05$, two-tailed, Cochran *t*-test) by 30 min. Glucose uptake remained depressed after 72 hr with continuous exposure to 10^{-8} M TCDD (data not shown).

Tables 3 and 4 present data from a series of experiments designed to investigate whether TCDD-induced reduction in glucose transport involves the Ah receptor, a cytosolic TCDD receptor and nuclear transcription factor known to regulate transcription of a number of genes by HAHs. We first investigated whether 4,7-phenanthroline, an Ah receptor blocker (*15*), could prevent TCDD from decreasing glucose uptake. The results shown in Table 3 indicate that this Ah receptor blocker actually increased glucose uptake. However, the effect of TCDD was minimal in cells treated with 4,7-phenanthroline as compared to those untreated with this blocker. In Table 4 the ability of two PBC congeners, known to differ in their activity as an agonist for the Ah receptor (*16*), were compared to TCDD for their ability to

inhibit glucose uptake. As expected, TCDD was the most potent inhibitor of specific glucose uptake followed by 3,3′,4,4′- and 2,2′,5,5′-tetrachlorobiphenyl (both tested at 10^{-7} M). The rank order of inhibition of glucose uptake matches the rank order for these compounds as Ah receptor agonists.

The combined actions of TCDD and a series of different hormones on glucose uptake are presented in Table 5. These glucose uptake assays were preformed on separate days using separate groups of differentiated adipocytes. Data presented in Table 5 are corrected for nonspecific binding of ^{3}H-Me-glc. The thyroid hormones T_3 and T_4, TNFα, TCDD, and the antibody against TNFα all significantly ($p \leq 0.05$, two-tailed Student's *t*-test) reduced glucose uptake over control. T_4 and the antibody against TNFα abolished the action of TCDD to further reduce glucose uptake. TNFα did not prevent a significant ($p \leq 0.05$, two-tailed, Student's *t*-test) TCDD-induced reduction in glucose uptake; nevertheless, the magnitude of TCDD-mediated decrease was not as great as for control and TCDD alone groups.

Table 5. Combined action of TCDD and some hormones on glucose uptake after 72-hr treatment of NIH 3T3 L1 adipocytes

Hormone	Total glucose uptake (pg 3-*O*-methyl-D-[1-^{3}H] glucose/1.5 million cells)[a] Control	TCDD
Assay 1[b]		
No addition (acetone vehicle)	16.9 ± 1.5	2.1 ± 0.2*
T3	5.0 ± 0.1**	0.5 ±1.3†
TNFα	5.8 ± 1.7**	0.5 ± 0.3†
Assay 2[b]		
No addition (acetone vehicle)	27.1 ± 2.6	2.9 ± 0.4*
T_4	0.3 ± 1.3**	0.4 ± 0.3
Antibodies against TNF	3.7 ± 1.7**	7.7 ± 0.3
TNFα	16.2 ± 1.5**	6.5 ± 0.5†

[a] 30-min incubation; means ± 1 SD; $n = 3$.
[b] The combined action of TCDD and hormones was investigated in two separate series of experiments. Hormone concentrations in maintenance media were 1 μg/ml epidermal growth factor, 1 ng/ml T_4, 10 ng/ml T_3, 10 ng/ml tumor necrosis factor-α (TNFα), and 1 μg/ml of antibody against TNFα. Cells were differentiated as described in Materials and Methods and then media changed. Maintenance media for treated cells contained 10^{-8} M TCDD with or without hormones, and cells were exposed to TCDD and hormones for 72 hr before the glucose uptake assay. Glucose uptake assay contained 1 μCi ^{3}H-Me-glc plus 13.3 mM cold glucose.
*TCDD-treated cells had significantly lower ^{3}H-Me-glc uptake than the corresponding control (no addition) ($p \leq 0,05$, two-tailed Student's *t*-test).
**Hormone-treated cells had significantly lower ^{3}H-Me-glc uptake than the coresponding no addition ($p \leq 0.05$, two-tailed Student's *t*-test).
†The effect of TCDD was significant ($p \leq 0.05$, two-tailed, Student's *t*-test) as compared to the matched cell preparation treated with a hormone only.

Discussion

Understanding of the process of glucose transport and its regulation has rapidly advanced over the last several years (*17,18*). The NIH 3T3 L1 preadipocyte cell line has served as a model system for investigations into glucose transport and was chosen for these investigations both for its widespread use as an *in vitro* model of adipose tissue function and because of its origin from TCDD-responsive Swiss albino mice. Initial experiments indicated TCDD inhibited glucose transport in 3T3 L1 adipocytes (Table 1). We have been interested in the key question of how TCDD is regulating glucose uptake and what types of glucose-transporting mechanisms TCDD affects. If cytochalasin B, a specific inhibitor of the facilitative glucose transporters GLUT 1 and GLUT 4 present in differentiated 3T3 L1 cells (*10,19,20*), and TCDD were acting on separate glucose-transport mechanisms, the affects of TCDD and cytochalasin B could be expected to be additive. However, they are not. The level of reduction of ^{3}H-Me-glc uptake is always the same magnitude for both TCDD and cytochalasin B treatment, and it could not be decreased further by treatment of cells with a combination of both of these agents. When nonspecific binding of ^{3}H-Me-glc was determined with cytochalasin B and corrected for, the inhibition of glucose uptake by TCDD became highly significant. ^{3}H-Me-glc uptake values then changed from about 15% inhibition by TCDD to about 90% inhibition for values corrected for nonspecific binding.

TCDD consistently inhibited glucose transport in 3T3 L1 adipocytes in a time- and dose-dependent manner, the effects of TCDD becoming statistically significant at a dose of 10^{-9} M (Fig. 1) and at 30 min (Fig. 2). The total percent inhibition of glucose uptake did vary between experiments and may be related to two factors: state of differentiation and length of time the cells were in culture after differentiation. The 3T3 L1 cell line is propagated as an undifferentiated preadipocyte, which has a fibroblastlike morphology. The cells are induced to differentiate by adding high levels of insulin, dexamethasone and methylisobutyl xanthine to the cell media. We observed variation in the extent of differentiation of preadipocytes into adipocytes between different replicates of cells. For replicates containing larger numbers of fully differentiated cells (cells containing large lipid storage vacuoles), the values for specific glucose uptake were greater. Length of time in culture after differentiation of the cells may also have been a factor because insulin alters glucose transporter number and function (*21*), and chronic treatment of 3T3 L1 adipocytes with insulin results in the downregulation of the GLUT 4 in 3T3 L1 cells (*22*). For reasons discussed below, GLUT 4 may be important for the effects of TCDD.

With regard to the nature of GLUT affected by TCDD, we compared the actions of TCDD in differentiated and undifferentiated cells to determine which of the two facilitative glucose transporters, GLUT 4 or GLUT 1, is affected by the actions of TCDD. In undifferentiated cells, GLUT 1 is the only glucose transporter present (*19*), whereas differentiation of these cells by exposure to insulin, dexamethasone, and methylisobutyl xanthine causes the induction of the GLUT 4 form of the facilitative glucose transporter to levels higher than that of GLUT 1 (*20*). When differentiated and undifferentiated cells were compared, the percent inhibition of glucose uptake by TCDD (Table 2) was larger for differentiated cells, being about 90% versus 40% for undifferentiated cells. Additionally, the total quantity of glucose uptake was increased by differentiation. Percent inhibition of glucose transport by TCDD was greater in the presence of 13.3 mM cold D-glucose than in the absence of cold D-glucose. Of the two GLUTs present in differentiated 3T3 L1 cells, GLUT 4 has a lower glucose affinity (K_m = 5 mM) than GLUT 1 (K_m<1 mM) (*23*). The results shown in Table 1 are consistent with the idea that TCDD produces a greater reduction in the quantity of the low affinity, GLUT 4, form of the glucose transporter. Results in Table 2 show that GLUT 4 is more susceptible to the action of TCDD, though GLUT 1 is also affected by TCDD. Data from *in vivo* and *in vitro* explant tissue culture studies in guinea pig adipose tissue are consistent with TCDD acting to reduce the number of GLUT 4 transporters (*7,8*).

The best-characterized mechanism for the action of TCDD involves the recently cloned Ah receptor (*24*), cytosolic receptor that binds TCDD and other HAHs and is then translocated to the nucleus, where it acts as a transcription factor to regulate gene expression (*25*). The reduction of glucose uptake in 3T3 L1 adipocytes in response to active and inactive congeners of tetrachlorobiphenyls (Table 4) correlates with other biological responses to dioxins (e.g., ethoxyresorufin *O*-demethlyase activity), which are mediated through the Ah receptor (*16*). Furthermore, such an affect of TCDD is antagonized by an Ah receptor blocker, 4,7 phenanthroline (*15*) (Table 3). Our data are also consistent with the results found *in vivo* and *in vitro* for guinea pig adipocyte tissue in which

reduction in glucose uptake correlated with the relative affinity of several dioxin congeners toward the Ah receptor (*7*).

The similarity of the action pattern of TCDD to thyroid hormones (*25*) has already been noted. We were interested in examining the effect of thyroid hormones on glucose transport alone and in combination with TCDD to ascertain if these two agents might share a common mechanism in 3T3 L1 cells. We found that the thyroid hormones T_3 and T_4, like TCDD, significantly reduced glucose uptake in differentiated 3T3 L1 cells (Table 5). In contrast, studies by Kuruvulla et al. (*26*) found that T_3 stimulates glucose uptake and increases GLUT 1 mRNA in a nontransformed liver cell line, and studies by Casla et al. (*27*) found stimulation of glucose uptake and increased levels of GLUT 4 protein in muscles of hyperthyroid rats. However, these studies were in different tissues and, especially in the case of muscle tissue, hyperthyroidism increases general glucose requirements. Since hyperthyroidism reduces adipose tissue mass, differences in glucose transport between 3T3 L1 cells and liver cells or muscle may be based on differences in organ responses to the thyroid hormones.

We did observe that T_4, and to a lesser extent T_3, antagonized the ability of TCDD to further reduce glucose uptake beyond the level already achieved by these thyroid hormones. Though these studies with hormones do not prove that TCDD and any of the hormones are acting on the same site or through the same mechanism, they are consistent with observations that TCDD and the thyroid hormones share similar effects and might share some common biochemical mechanisms. Clearly, the titer of functionally active glucose transporter proteins are readily modulated by these hormone-type substances as well as TCDD.

In summary, the present study extends previous *in vivo* and *in vitro* work documenting the TCDD-mediated inhibition of glucose uptake (*7,8*). Furthermore, it has established the sensitivity of GLUT 4 to TCDD using the *in vitro* NIH 3T3 L1 preadipocyte model system. Experimental evidence is consistent with TCDD inhibiting glucose uptake by regulation of GLUT 4 and, to a lesser extent, GLUT 1 through a mechanism involving the Ah receptor.

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